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Human Pathogenic Arenaviruses (*Arenaviridae*)

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History and Classification

The first arenavirus, lymphocytic choriomeningitis virus (LCMV), was isolated in 1933 from a suspected case of St. Louis encephalitis. Shortly after LCMV was found to cause aseptic meningitis in humans, and the house mouse was identified as its natural reservoir. By the late 1960s the *Arenaviridae* family was recognized to include a group of viruses that shared with LCMV common morphology, serology, biochemical features, and a natural history of establishing long-term chronic infections in their natural rodent hosts. The pathogenic potential of arenavirus infections in humans was underscored with the discovery of Junín virus (JUNV) in the late 1950s as the causative agent of Argentinian hemorrhagic fever (AHF), and the subsequent identification of Machupo virus (MACV) and Lassa virus (LASV) in 1962 and 1969 as the etiologic agents of Bolivian hemorrhagic fever (BHF) and Lassa fever (LF), respectively. In the following years, additional pathogenic arenaviruses were discovered, including Guanarito (GTOV), Sabiá (SBAV), and Chapare (CHAPV) viruses in South America, and Lujo virus (LUJV) in Africa. During the last decade the use of the new technologies of deep sequencing and genome discovery has resulted in the discovery of many new arenaviruses, but their contribution to human disease remains to be determined. Notably, arenaviruses have been also discovered in reptiles (snakes) and fish, representing a significant expansion of the host range of arenaviruses.

Current arenavirus classification is based on pairwise sequence comparisons (PASC) of coding-complete genomes. Based on the most current sequence dataset, S segment and L segment nucleotide sequence identities for viruses within the same genus need to be higher than 40% and 35%, respectively, This analysis and additional characteristics, such as genome architecture, host range, modes of transmission and/or sites of replication in the cell, led to the establishment of four genera within the family *Arenaviridae*: *Antennavirus*, *Hartmanivirus*, *Mammarenavirus*, and *Reptarenavirus*. The hosts of mammarenaviruses are rodents, with the exception of Tacaribe virus (TCRV) that has been found in phyllostomid bats and ixodid lone star ticks. Reptarenaviruses and hartmanivirus infect reptilian hosts, and these infections can cause boid inclusion body disease (BIBD). Antennaviruses have a piscine host. Based on antigenic properties, mammarenaviruses have been divided traditionally into two distinct groups. Old World (OW) mammarenaviruses ("Lassa–LCMV serocomplex") include viruses indigenous to Africa and the worldwide distributed LCMV, whereas New World (NW) mammarenaviruses ("Tacaribe serocomplex") include viruses indigenous to the Americas. This classification is largely consistent with phylogenetic data and muroid rodent host phylogeny, with OW mammarenaviruses infecting murid rodents primarily in Africa and NW mammarenaviruses infecting cricetid rodents primarily in the Americas.

This article focuses on arenaviruses known to cause disease in humans, all of them within the *Mammarenavirus* genus.

Mammarenavirus Virion Structure

Mammarenavirions are pleomorphic, ranging in size from 40 to more than 200 nm in diameter, with dense lipid envelopes. The virion's surface is decorated with evenly spaced spike projections composed of heterotrimer complexes of the viral glycoproteins GP1 and GP2 and the stable signal peptide (SSP) (**Fig. 1(A)**). Cryo-EM studies have shown that surface GP complexes are aligned with subjacent Z and ribonucleoprotein (RNP) densities, which are packed into a two-dimensional lattice at the inner surface of the viral membrane. Virions contain the L and S RNP complexes organized in circular configurations. The L and S genomic RNAs are not present in equimolar amounts within virions (L:S ratios $\sim$ 1:2), and low numbers of both L and S antigenomic RNAs are also present within virions. In addition, host ribosomes are documented to be incorporated into virions, but the biological significance of this incorporation remains uncertain.

Mammarenavirus Genome Organization and Proteins

Mammarenaviruses have a bi-segmented negative-stranded RNA genome (**Fig. 1(B)**). Each genome segment, L (large, 7.3 kb) and S (small, 3.5 kb) uses an ambisense coding strategy to direct the synthesis of two proteins from two non-overlapping open reading frames (ORF) of opposite polarities that are separated by non-coding intergenic regions (IGRs). The S RNA encodes the viral NP and the glycoprotein precursor (GPC), whereas the L RNA encodes for the viral RNA dependent RNA polymerase (RdRp) (L protein) and the matrix Z protein.

Mammarenavirus genomes exhibit high degree of sequence conservation at their 3′-termini and similarly to other viruses with segmented negative strand (sNS) RNA genomes, they exhibit 5′- and 3′-inverted complementary sequences at their L and S genome segments that are predicted to form panhandle structures. This prediction is supported by EM data showing the existence of circular RNP complexes within arenavirions. For several arenaviruses, a non-templated G residue has been detected at the 5′ end of progeny genomic RNAs during replication. The IGRs are predicted to fold into stable hairpin structures. Transcription of the

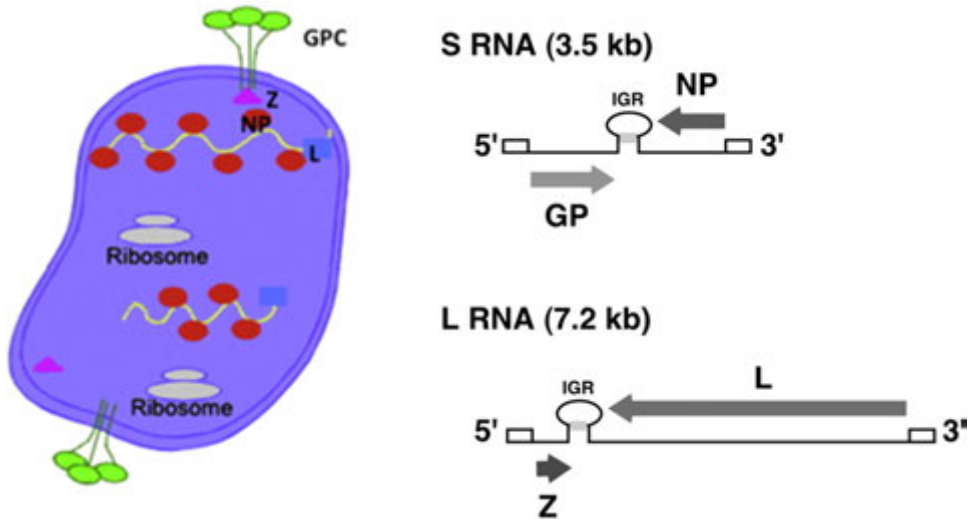

Fig. 1 A. Schematic illustration of an arenavirus particle. Shown is the pleomorphic shape of the enveloped virion particle decorated by spikes composed of heterotrimers of GP1 and GP2 associated with the SSP (no shown in the figure). The surface glycoproteins are aligned with subjacent matrix Z protein that serves also as a bridge between the surface glycoproteins and the S and L vRNP complexes inside the particle. Each vRNP consists of the NP (red) and L polymerase (blue) together with the S or L genome RNA species. Mammarenavirus virions are thought to incorporate ribosome particles. B. Mammarenavirus genome organization. Each genome segment uses an ambisense coding strategy to direct the synthesis of two different proteins in opposite direction and separated by a non-coding intergenic region (IGR) that serves as a bona fide transcription termination signal. The S segment encodes for the virus NP and GPC precursor, whereas the L segment encodes for the L polymerase and the matrix Z protein.

S–derived NP and GP mRNAs was shown to terminate at multiple sites within the predicted distal stem of the IGR, supporting the view that a structural motif rather than a sequence-specific signal promotes the release of the arenavirus polymerase from the template RNA. There are significant differences in sequence and predicted folded structure between the S and L IGRs, but among isolates and strains of the same arenavirus, the S and L IGR sequences are highly conserved. In addition to their role in control of transcription termination, IGRs have been shown to play a critical role in production of infectious particles.

GPC is co-translationally cleaved by the signal peptidase to generate a 58-amino acid stable signal peptide (SSP), and post-translationally processed by the cellular protease subtilisin kexin isozyme-1 (SKI-1)/ site 1 protease (S1P) to generate the mature virion surface glycoproteins GP1 and GP2. GP1, GP2 and SSP form the GPC complexes that decorate the surface of virions and mediate virus receptor recognition and cell entry. GP1 mediates virion interaction with host cell-surface receptors, whereas GP2 directs fusion of virus and host cell membranes. The fusion process depends on a low pH-driven conformational change of GP2 from a metastable prefusion structure to a more stable postfusion six-helix bundle. Notably, the SSP contributes to trafficking and processing of GPC as well as to the GP2-mediated pH-dependent fusion process.

NP is the most abundant viral polypeptide both in infected cells and virions. It is the main structural component of the viral RNP responsible for directing RNA genome replication and gene transcription. The C-terminus of NP exhibits a type I interferon (IFN-I) counteracting activity. Crystallographic studies identified distinct N- and C-terminal domains within NP of LASV, LCMV and JUNV, features that are expected to be present in other mammarenavirus NPs. The N-terminal domain of NP contains an RNA-binding site and plausible cap-binding activity. The C-terminal part of NP contains a functional DEDDH 3′–5′ exoribonuclease folding domain similar to the one described for the non-structural 14 protein of severe acute respiratory syndrome (SARS) coronavirus. This $3' - 5''$ exoribonuclease activity of NP plays critical roles in NP's anti-IFN activity and in other additional steps of mammarenavirus multiplication yet to be elucidated.

Mammarenavirus L proteins have a central region that includes conserved motifs characteristically found in the RdRp domains of negative-sense RNA viruses. Residues critical for LASV L function have been shown to be located both within and outside the predicted RdRp domain. Bioinformatic analysis and structural studies revealed that the N-terminus of LASV L has an endonuclease domain of similar structure to the cap-snatching endonuclease domains of influenza A virus polymerase acidic (PA) and La Crosse virus L proteins. In addition, EM characterization of a functional MACV L has revealed a core ring-domain decorated by appendages, which likely reflects a modular organization of the arenavirus polymerase.

Mammarenavirus Z proteins exhibit a modest degree of overall conservation. However, three regions within Z have a higher degree of conservation: (1) an N-terminal myristoylation site marked by a highly conserved G motif that is critical for membrane anchoring and interaction with other viral proteins; (2) a RING domain that binds two zinc ions through three conserved motifs; and (3) a C-terminal region containing proline-rich motifs that serve as functional late budding motifs. Structural studies revealed that the N- and the C-terminal arms flanking the RING domain are disordered, which may enable Z to recruit a variety of cellular partners to modulate virus multiplication. In addition, Z assembles in a dodecameric manner through a head-to-tail dimerization of the RING domain. These findings are consistent with Z playing a key role in virion assembly, which is further supported by EM

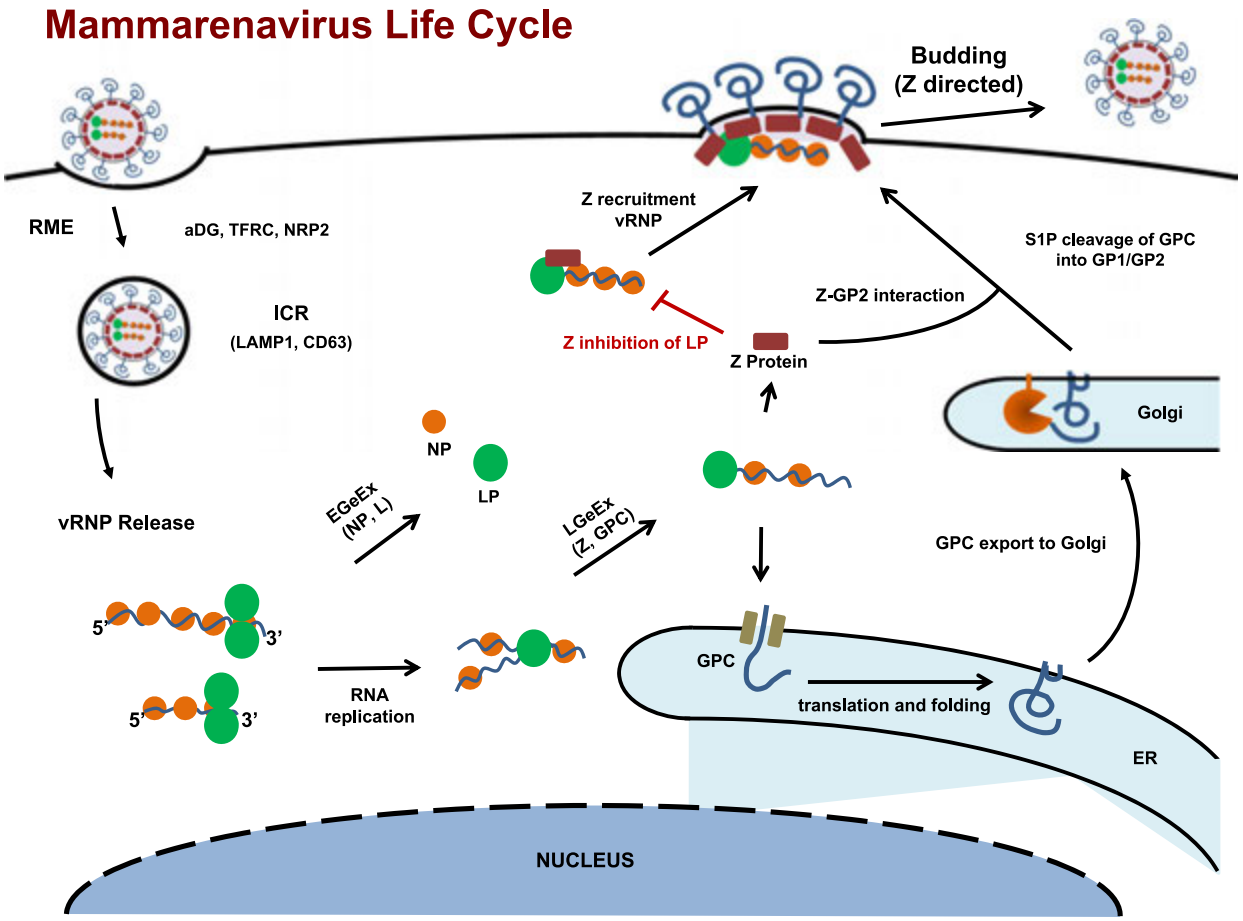

Fig. 2 Mammarenavirus life cycle. Mammarenavirus enter cells via receptor-mediated endocytosis (RME). There are some differences on the specific steps of the pathway used by different mammarenaviruses to reach the late endosome where the acidic environment triggers a pH-dependent fusion event between viral and cell membranes that releases the vRNP into the cytoplasm of the infected cells where viral RNA replication and gene transcription take place. Correct processing of GPC by the cellular signal peptidase and S1P protease is required for the formation of infectious progeny. The matrix Z protein plays critical roles mediating interactions between the vRNP and surface GP complex required for the formation of infectious progeny. Z is also the main driving force of mammarenavirus budding.

studies indicating that Z bridges the viral RNP to the viral envelope proteins. Z is also involved in suppression of the host cell type I IFN response via its interaction with RIG-I-like receptors.

Mammarenavirus Life Cycle

Cell Attachment and Entry

Receptor-mediated endocytosis is the main cell entry pathway used by mammarenaviruses. The acidic environment of the late endosome facilitates a pH-dependent conformational change in the GP complex and subsequent GP2-mediated fusion step between viral and cell membranes. Following fusion, the viral RNP is released into the cytoplasm where it directs both replication and transcription of the viral genome (Fig. 2). The conserved and widely expressed cell-surface receptor for extracellular matrix proteins α-dystroglycan (aDG) is a main receptor for the OW mammarenaviruses LCMV and LASV. Posttranslational glycosylation modification of aDG by the like-acetylglucosaminyltransferase (LARGE) is critical for aDG's function as a mammarenavirus receptor, and specific LARGE alleles have been shown to be positively selected among the Yoruba of Western Africa where LASV is endemic. However, non-pathogenic mammarenaviruses also use aDG-mediated cell entry, indicating that aDG does not play a unique direct role in mammarenavirus pathogenesis. Secondary alternative receptors, including members of the Tyro3/Axl/Mer and T-cell immunoglobulin (TIM) and mucin receptor families may account for LASV and LCMV infection of cells lacking fully glycosylated aDG. Cell entry of the OW hemorrhagic fever (HF) mammarenavirus LUJV is mediated by neuropilin (NRP)-2, a cell-surface receptor for semaphorins. NRP-2 is highly expressed in microvascular endothelial cells, which may contribute to LUJV-induced coagulopathy. Human transferrin receptor 1 (TfR1) is the main cellular receptor used for cell entry of pathogenic NW mammarenaviruses, including JUNV and MACV. Consistent with the use of TfR1 as a primary receptor, JUNV enters cells through clathrin-mediated endocytosis.

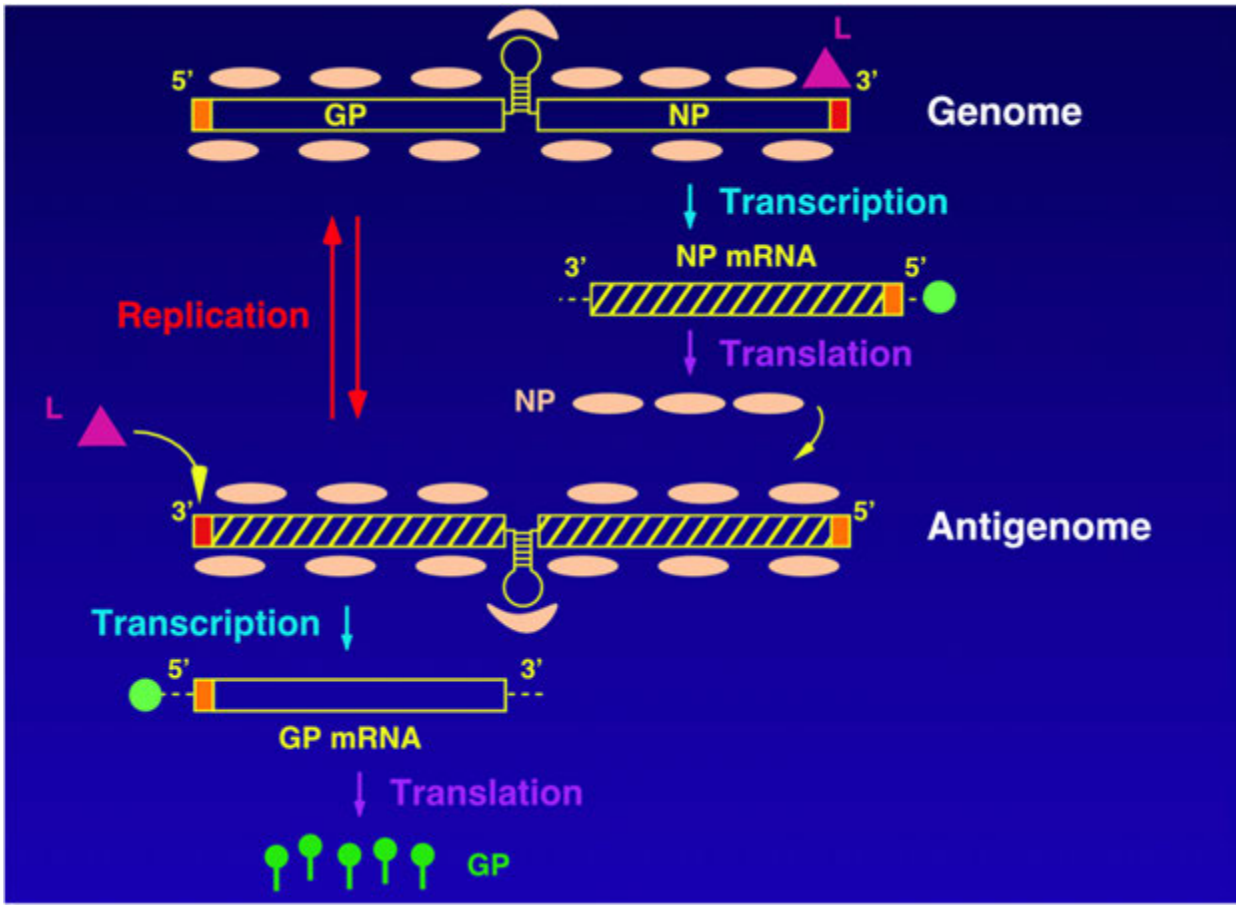

Fig. 3 Mammarenavirus RNA replication and gene transcription. The basic steps of mammarenavirus RNA replication and gene transcription are illustrated for the S segment. Following the pH-dependent fusion event between the viral and cell membranes in the late endosome, the vRNPs are delivered in to the cytoplasm where they initiate transcription from the genome promoter located at the genome 3′-end. Primary transcription results in synthesis of NP and L mRNA from the S and L segments, respectively. Subsequently, the virus polymerase adopts a replicase mode and moves across of the IGR to generate copies of the full length antigenome RNA (agRNA) species that serve as template for the synthesis of the GPC (agS) and Z (agL) mRNAs. The agRNA species serve also as templates for the amplification of the corresponding genome RNA species.

Notably, completion of the cell entry process for LASV and LUJV involves a late endosomal receptor switch mechanism. LASV uses the late endosomal resident proteins LAMP1 and LUJV uses CD36 in these final entry stages.

Expression and Replication of the Viral Genome

NP and L coding regions are transcribed into a genomic complementary mRNA. However, the GPC and Z coding regions are not translated directly from genomic RNA, but rather from genomic sense mRNAs (Fig. 3). These genomic sense mRNAs are transcribed from templates of the corresponding antigenome RNA, which also function as replicative intermediates. Transcription initiation is carried out through a mechanism shared by all negative sense viruses called cap-snatching, in which short fragments of 5′-capped host cell mRNAs are used by virus polymerase complex to prime the synthesis of viral mRNAs. Transcription termination occurs within the distal side of the IGR and generates non-polyadenylated viral mRNAs. Virus replication proceeds in two steps: first, the *de novo* (primer-independent) synthesis of an intermediate RNA with positive polarity (cRNA) and second, using this cRNA as template, the synthesis of progeny genomic vRNA with negative polarity. The 5′-end of arenavirus genome and antigenome RNAs each contain a non-templated G residue that has been proposed to reflect a prime-and-realign mechanism for RNA replication mediated by L.

Using cell-based minigenome (MG) assays, NP and L have been identified as the minimal viral *trans*-acting factors required for efficient RNA synthesis mediated by the virus polymerase. For LCMV, both genetic and biochemical evidence indicate that oligomerization of L is required for polymerase activity. Z is not required for RNA replication and gene transcription mediated by the virus L polymerase, but rather exhibits a dose-dependent inhibitory effect on both RNA biosynthetic processes. Consistent with these findings, studies using in vitro reconstitution of RNA synthesis directed by MACV polymerase have provided evidence that Z, via direct interaction with the polymerase, is able to lock the polymerase in a promoter-bound, catalytically inactive state.

Mutation-function analysis of the genome 5′-and 3′-termini using cell-based MG assays for LCMV, LASV, and MACV indicate that the activity of the arenavirus genomic promoter is dependent on both sequence specificity within the highly conserved 3′-terminal 19 nt of arenavirus genomes and on the integrity of the predicted panhandle structure. This panhandle is formed via sequence complementarity between the 5′- and 3′-termini of viral genome RNAs. Mammarenavirus RNA replication and gene transcription are regulated in a coordinated manner but intracellular levels of NP do not determine the balance between virus RNA replication and transcription. MG-based assays confirmed the IGR role as a *bona fide* transcription termination signal, but synthesis of translation-competent viral mRNAs does not strictly require the presence of the IGR.

Assembly and Budding

Production of infectious mammarenavirus progeny requires both Z and GPC and the correct processing of GPC into GP1 and GP2. Z is a structural component of the virion and cryo-electron microscopy structural studies reveal a location of LMCV Z within virions consistent with its role as a matrix protein. Consistent with its matrix protein function, Z is the main driving force of mammarenavirus budding, a process mediated by the interaction of Z's late domain budding motifs, PTAP or PPPY, or both, with components of the endosomal sorting complexes required for transport (ESCRT) within the vacuolar protein sorting (Vps) pathway. Myristoylation of Z is strictly required for its targeting to the plasma membrane, the location of arenavirus budding (**Fig. 2**).

Epidemiology of Human Pathogenic Mammarenaviruses

Typically, mammarenaviruses cause persistent, frequently asymptomatic infections in their reservoir hosts, which are characterized by virus multiplication in many different tissues and chronic viremia and viruria. Both, vertical transmission (exposure to infectious virus early in ontogeny) and horizontal transmission (exposure to virus later in life through aggressive or venereal behavior) may contribute to the mammarenavirus chronic carrier state in rodents. Human infections occur via exposure to rodent fomites, ingestion of contaminated food, exposure to broken skin or mucous membranes, or by inhalation of aerosolized virions from contaminated material.

Lassa Fever (LF)

LF is caused by LASV, which is endemic in vast areas of western sub-Saharan Africa. Imported cases of LF have been reported in the United States, Canada, the United Kingdom, Japan, Germany, Netherlands, and Israel. The case-fatality rate (CFR) of LF is about 1%–2% in the endemic areas, with estimated 300,000 infections annually. Most LASV infections in Africa are asymptomatic, mild or subclinical, but the CFR in hospitalized confirmed cases of LF can be as high as 69%. The disease is especially severe late in pregnancy with fetal death, miscarriage, or spontaneous abortion occurring in nearly all cases.

The main reservoir host of LASV is the Natal multimammate mouse (*Mastomys natalensis*). Maintenance of LASV in Natal mastomys is thought to occur via vertical transmission but recent studies suggest the existence of an additional horizontal transmission mechanism. Other rodents might also serve as LASV hosts or have roles in LASV transmission. Humans become infected with LASV through direct contact with infected rodent tissues, excreta, or blood or via inhalation of aerosolized virus. Infected meat could contribute to virus transmission in populations that include peridomestic rodents as part of their diet. Person-to-person transmission of LASV can occur in nosocomial settings via direct contact with body fluids from symptomatically infected individuals or corpses. Human infections tend to be more common in the dry season, which may reflect a higher prevalence of Natal mastomys within human dwellings. LASV prevalence is focal and varies greatly between geographical regions. For example, in Sierra Leone, seroprevalence ranges from 8% to 52% and in Nigeria from 13% to 37%.

LASV exhibits a high degree of genetic diversity; nucleotide differences can reach up to 32% for the L segment and 25% for the S segments. The genetic diversity among LASV strains correlates with geographic distribution rather than time. This high genetic diversity of LASV might explain the observed variability of LF's clinical presentation and possible regional differences associated with CFRs and disease symptoms.

Lujo Hemorrhagic Fever (LHF)

LHF, caused by LUJV, was identified in 2008 in South Africa. The only known nosocomial outbreak involved five cases of viral HF disease with clinical symptoms remarkably similar to LF, despite LASV and LUJV being distantly related. LHF disease has an abrupt onset and exhibits disseminated intravascular coagulation (DIC), features that distinguish LHF from LF. The overall lack of epidemiological data on the prevalence of LUJV in South Africa and the limited number of verified cases prevents any conclusion on whether the high (80%) CFR associated with the LHF outbreak reflects a more severe disease compared to LF. The natural reservoir of LUJV remains unknown.

Lymphocytic Choriomeningitis (LCM)

The primary host of LCMV is the house mouse. Other rodents, including pet hamsters and domesticated guinea pigs can become infected and transmit the virus to humans. LCMV is transferred vertically from one generation to the next and chronically infected

rodents shed the virus in their urine, saliva, nasal secretions, and droppings throughout life. Mounting evidence indicates that LCMV is a neglected human pathogen of clinical relevance as an under-recognized cause of neurologic disease in the fetus, and adult, and important threat to immune compromised individuals. Humans become infected with LCMV through close contact with infected rodents, solid organ transplantation, or by vertical transmission associated with congenital infections. The seroprevalence of LCMV within house mouse populations and in humans is highly variable, even within the same geographic region, resulting in the focal and uneven spatial distribution of the virus. As with LASV, LCMV exhibits high genetic diversity. Up to 18% and 25% nucleotide divergence was observed within the S and L segments, respectively, of LCMV lineages. The high genetic diversity of LCMV and the lack of clear correlation of virus genetic lineages to particular geographic locations likely reflect the long and complex phylogeographic history of the common house mouse host.

Argentinian Hemorrhagic Fever (AHF)

Junín virus (JUNV), the etiologic agent of AHF, is endemic to the Pampas in Argentina. In the absence of treatment, the CFR rate can reach 20%–30%. Pregnant women have higher CFR and many miscarry, especially if infection occurs during the third trimester. AHF is typically a seasonal disease, with a peak of frequency occurring during the corn-harvesting season, and infected cases are primarily rural male agricultural workers. The drylands laucha (*Calomys musculinus*) is the main reservoir of JUNV but other animals can also become infected. The patchy spatial distribution of the drylands laucha has been suggested to account for the focal distribution of AHF. Annual increase in the number of these rodents coincides with the corn harvesting season providing opportunities for rodent-to-man transmission. Human transmission is thought to occur predominantly by inhaling aerosolized viral particles from contaminated soil and plant litter, which are disturbed during the mechanized harvesting process, or by exposure to primary aerosols of rodent urine or contact with contaminated nesting materials in border habitats. Horizontal transmission via aggressive encounters among adult, male rodents is the primary mode of viral persistence in nature, which is facilitated by the high viral load in saliva of drylands lauchas. Vertical transmission might contribute to the maintenance of JUNV via intra-generational infection by horizontal transmission when population numbers are reduced. Seroprevalence in humans range from 4.7% to 12.3% in AHF-endemic areas, and significantly lower (0.44%) in non-endemic areas. In rodents, prevalence of infection can be as high as 10.9% with highest prevalence observed in current epidemic areas and lowest (0.2%) in non-endemic areas. In contrast to LASV and LCMV, Nucleotide similarity between JUNV strains is high, reaching up to 94.5% and 95.4% for GP and NP, respectively.

Bolivian Hemorrhagic Fever (BHF)

The CFR of BHF, caused by Machupo virus (MACV), is approximately 5%–30% with the highest rates occurring among those under 5 and over 55 years of age. BHF cases are more common at the peak of agricultural activity, during the dry season, with males over 15 years of age more frequently affected. The big laucha (*Calomys callosus*) is the reservoir host of MACV. Both horizontal and vertical transmission have been shown as possible maintenance mechanism of MACV within its reservoir rodent. Person-to-person transmission of MACV is possible but rather uncommon, likely reflecting the infrequent and low virus detection in blood or from throat and oral swabs of infected patients.

Venezuelan Hemorrhagic Fever (VeHF)

Guanarito virus or GTOV emerged in 1989 as the cause of VeHF, a severe hemorrhagic illness with CFR of $\approx$30%. The disease has focal distribution in the southern and southwestern portions of Portuguesa state and in adjacent areas of Barinas state. VeHF cases peak during the period of agricultural activity in these regions and involve mainly male agricultural workers. The short-tailed zygodont (*Zygodontomys brevicauda*) is the main reservoir host of GTOV. The virus is also commonly found in Alston's cotton rats (*Sigmodon alstoni*). These hosts have not been reported within houses or farm building. Therefore, infections are assumed to occur outdoors, in rural areas, and persons that have frequent contact with rodent-infested grassland habitats are at higher risk.

Pathogenesis and Pathology

The mechanisms underlying pathogenesis of mammarenaviral disease in humans are not well understood. Pathological findings in patients' autopsies do not account for the severity of symptoms seen in many cases of human mammarenaviral disease. Mammarenaviruses typically enter humans in an aerosolized form and are deposited in the lung, where initial viral replication occurs. Antigen-presenting cells (APCs), DCs and macrophages, are prominent targets in the initial stages of infection, and facilitate virus access to the lymphoid system and subsequent systematic spread to other organs and tissues including liver, kidneys, lungs, adrenal glands, and heart. LASV has also been recovered from placenta, mammary glands, and aborted fetal tissues, and in cases of AHF, virion-like particles were detected in the central nervous system (CNS), ovaries, and testes. LCMV load can reach high levels in meninges, choroid plexus, and ventricular ependymal linings, where the inflammatory response produces the characteristic LCM pathology. LCMV exhibits a strong tropism for the fetal brain, where LCMV congenital infection produces its

most common and severe pathologic effects, including microencephaly, periventricular calcifications, hydrocephalus, cerebellar hypoplasia, focal cerebral destruction, and gyral dysplasia.

The extent of hemorrhagic and coagulopathy manifestations differs between human pathogenic mammarenaviruses. Hemorrhages are common in AHF cases, but are rare and mainly limited to mucosal surfaces in LF cases. Bleeding is usually associated with thrombocytopenia and platelet dysfunction. Fatal cases of LF are associated with lower levels of platelet activating factor (PAF) and PAF-like molecules, as well as hemoglobin breakdown products D-urobilinogen and I-urobilin. Disseminated intravascular coagulation (DIC) or complement activation do not appear to play a role in mammarenavirus pathogenesis.

Impaired vascular endothelium function, including increased permeability, likely plays a central role in LF and AHF pathogenesis. However, only minimal vascular histological lesions are detected in fatal human LF and AHF cases and infected nonhuman primates (NHPs), and the mechanisms responsible for virus induced increase in vascular permeability remain to be elucidated. In contrast to other HF-causing viruses, LASV and JUNV do not trigger a "cytokine storm" that could interfere with the integrity of the vascular endothelium, but infection of the endothelium might cause changes in endothelial cellular function leading to increased fluid flow and subsequent edema.

Pathological findings in LF patients include both macroscopic and microscopic abnormalities. Gastric mucosal, renal, and subconjunctival hemorrhages, petechiae, and increased vascular permeability are common macroscopic observations, whereas microscopic observations include multifocal hepatocellular necrosis with modest inflammatory cell involvement, splenic necrosis, necrosis of renal tubular cells, adrenocortical cell necrosis, focal renal interstitial lymphocytic infiltrates, mild mononuclear interstitial myocarditis, alveolar edema with capillary congestion, interstitial pneumonitis, and rhabdomyositis.

The most consistent pathological hallmark of LF in humans is multifocal hepatocellular necrosis. High virus titers in liver tissue correlate with severe hepatitis. However, the degree of hepatic tissue damage is insufficient to cause hepatic failure, and there is no correlation between the degree of hepatic necrosis and chemical indicators of liver damage, indicating that LASV-induced hepatitis is unlikely to be the primary cause of death in fatal cases of LF.

The most common macroscopic abnormality observed in severe cases of NW mammarenaviral disease is widespread hemorrhage affecting the skin and mucous membranes, Virchow-Robin space, kidneys, pericardium, spleen, adrenal glands, and lungs. Microscopic lesions include acidophilic bodies, focal liver necrosis, acute tubular and papillary necrosis in the kidneys, and reticular hyperplasia of the spleen and lymph nodes. Pathognomonic symptoms of AHF illness can vary depending on the specific JUNV strain and can be "hemorrhagic", "neurologic", "mixed", and "common". Infection with "hemorrhagic" strains results in a pronounced bleeding tendency with disseminated cutaneous and mucous membrane hemorrhage. In contrast, infection with "neurologic" strains show little or no hemorrhagic manifestations but result in overt and progressive signs of neurologic dysfunction.

LCM disease correlates with detection of viral antigen in meninges and cortical neurons, as well as mononuclear cell infiltrates in the meninges and around vessels and glial nodules in the deeper structures, suggesting an immune-mediated pathology associated with LCMV infection. An animal model of congenital LCMV infection revealed a very strong tropism of LCMV for neuroblasts and altered neuron migration, findings that may explain the location of periventricular calcifications and the gyral malformations in children with congenital LCMV.

Clinical Features

LF and LHF

LF is mild or asymptomatic in about 80% of infected individuals, but 20% develop acute LF. The incubation period can range from 5 to 21 days but is typically 10–14 days. Within 2–4 days of infection, many patients experience an array of symptoms including headache, myalgia, arthralgia, lower back, abdominal and retrosternal chest pain, dizziness, nausea, tinnitus and sore throat. Cough, vomiting, diarrhea and constipation are also common, whereas lymphadenopathy, oliguria, tachycardia, vertigo, splenomegaly, hepatomegaly, and jaundice have been reported occasionally. Disease progression is often associated with pharyngitis, conjunctivitis, respiratory distress, pleural and pericardial effusions, and facial and neck edema. In most cases of LF recovery begins 8–10 days after disease onset, and 4–11 days later viremia is undetectable. In contrast, in severe cases of LF, worsening of symptoms and hemorrhagic and neurologic manifestations are more common. Hemorrhagic manifestations can affect skin and mucosal surfaces. Neuroglial findings including diffuse encephalopathy, confusion, tremors, coma, and convulsions are common prior to shock and death. Widespread edema can be observed in children with LF, which usually has a fatal outcome.

Common clinical findings in LF cases include proteinuria, albuminuria, and elevated AST levels, whereas moderate leukopenia has been reported only in some patients. Survivors of LF often recover without sequalae. However, long-term unilateral or bilateral sensorineural deafness can affect a significant ($\approx$13%–30%) number of survivors.

Known cases of LHF disease presented with similar clinical symptoms to those documented for LF cases. Initial symptoms of nonspecific febrile illness increase in severity over 7 days with the development of diarrhea, pharyngitis, and facial and neck edema. Terminal features are shock and multi-organ system failure often with evidence of DIC that resulted in death of four of the five LHF cases.

LCM

In most healthy individuals, LCMV infection course is asymptomatic, or manifested as a mild febrile illness. Following 1–2 weeks of incubation, some infected individuals may develop a flu-like illness that could include anorexia and gastrointestinal

symptoms. Sore throat, cough, pharyngitis, and other symptoms of respiratory tract involvement are less common. Leukopenia, moderate thrombocytopenia, mild elevations of AST, and infiltrates on chest radiographs can be seen in some patients. Additional, but rather infrequent, reported clinical findings are lymphadenopathy, dysesthesia, conjunctivitis, arthralgia, arthritis, rash, testicular or parotid pain, as well as abdominal, back, and chest pain. LCMV invasion of the CNS can occur in some infected individuals, usually resulting in symptoms of classic aseptic meningitis. In some cases, encephalitis, encephalomyelitis, meningoencephalitis, acute hydrocephalus, ascending or bulbar paralysis, or transverse myelitis may develop. CSF pleocytosis is characteristically observed during this CNS phase of illness and LCMV can be isolated from the spinal fluid. Infections are rarely fatal, and most patients recover without sequelae, but some might experience asthenia, headaches, confusion and difficulty in concentration.

LCMV Infection during pregnancy, especially during the first trimester, increases the risk for miscarriage, *in utero* fetal death, fetopathy, and severe neurologic sequelae. Congenital LCMV can result in severe CNS or ocular malformations and can mimic the signs/symptoms of classic TORCH syndrome. In immunosuppressed organ recipients, LCMV infection can be fatal due to multi-organ system failure with LCMV-associated hepatitis as a prominent feature. In most immunosuppressed patients CNS manifestations are also observed. Clinical findings in these patients include elevated transaminases levels, leukopenia, and thrombocytopenia, as well as increased protein levels and leukocyte numbers in CSF.

NW Mammarenaviral HFs

AHF has an incubation period of 1–2 weeks, followed by a prodromal phase characterized by fever and malaise, headache, myalgia, epigastric pain, and anorexia. After 2–4 days, signs become increasingly severe including prostration and GI disturbances. In some cases, dizziness, photophobia, retro-orbital pain, or disorientation may also occur. Initial signs of vascular damage may appear at this early phase of AHF. During the second week of illness, about 20%–30% of patients develop severe hemorrhagic and neurologic manifestations, or secondary bacterial infections. Hemorrhagic manifestations include bleeding from mucous membranes and ecchymosis at needle puncture sites, but only very modest blood loss is observed. Neurologic manifestations include seizures, convulsions, tremor of the hands and tongue, and less frequently, delirium, coma, encephalitis and meningoencephalitis. Following shock, death usually occurs 7–12 days after disease onset. Clinical laboratory findings include leukopenia and thrombocytopenia and non-specific electrocardiogram abnormalities, whereas chest radiography is usually normal in the absence of secondary infections. Patients' clinical improvement during the second week of AHF correlates with the appearance of neutralizing antibodies. Convalescence often lasts several weeks with polyuria, fatigue, alopecia, and dizziness.

BHF and VeHF caused by MACV and GTOV, respectively, as well as NW mammarenaviral HF caused by CHAPV and SBAV, display clinical symptoms similar to those described for AHF. Proteinuria and elevated hematocrit during the peak of hemorrhagic manifestations are characteristic of severe cases of BHF. Early symptoms of VeHF disease are indistinguishable from dengue fever, also common in Venezuela. CNS manifestations of VeHF including encephalitis are associated with poor prognosis. Hearing loss has been reported in convalescence cases of VeHF. The only reported case of a naturally acquired infection with SBAV caused disease with symptoms similar to those of other NW mammarenaviral HF, but also included extensive liver necrosis.

Diagnosis

LF

LASV can be isolated from blood during the febrile phase of LF disease, as well as from autopsy tissue samples. Serum detection of LASV antigen by ELISA is robust, reliable, and can be completed in a short time. LASV-specific antibodies can be detected by immunofluorescence (IF) and ELISA. ELISA IgM titers appear earlier and persist longer than IF IgM titers. Virus-specific IgG ELISA antibody detection persists for long periods, whereas IF antibody appears to wane over time below detectable limits. Reverse transcriptase-PCR (RT-PCR) can detect virus RNA in blood with high sensitivity. Nanopore sequencing using the MiniON device was successfully used to genetically characterize LASV isolates during the 2018 upsurge of LF cases in Nigeria. The ability of this portable sequencing technology to genetically characterize in situ RNA viral samples in real-time cases of LASV infection associated with disease symptoms will represent a major breakthrough in the study of LF epidemiology.

LF presents with symptoms indistinguishable from those of other febrile illnesses including malaria, and therefore it is difficult to diagnose LF clinically, but it should be suspected in patients with fever ($\geq 38^{\circ}C$) not responding to antimalarial and antibiotic drugs. Useful clinical findings for diagnosis of LF are fever, pharyngitis, retrosternal pain, and proteinuria. Viremia levels, fever, sore throat, vomiting, edema, and bleeding are the predictors of poor prognosis. CNS signs, face and neck edema, jaundice, bleeding, hematuria, and proteinuria were shown to be associated with a fatal outcome. Several biomarkers including increased levels of blood urea nitrogen and creatinine (biomarkers for kidney function), serum AST (liver malfunction), and serum electrolyte potassium, have been shown to have a strong correlation with CFR, highlighting the role of liver and renal dysfunction and electrolyte disturbance in the severity of LF.

LCM

LCMV can be isolated from blood during the febrile phase of LCM and during meningitis symptoms, but CSF contains higher viral load, and PCR-based tests using CSF samples have been successfully used. LCMV-specific IgM antibodies can be detected by ELISA and IF in serum and CSF in acute cases of LCM. Neutralizing antibodies appear late after onset of disease and their diagnostic value is limited.

NW Mammarenaviral HFs

During the acute febrile phase of disease, virus can be isolated from blood samples by inoculation of newborn hamsters or mice, but cocultivation of patient's peripheral blood mononuclear cells with Vero cells may offer higher level of sensitivity. Virus can also be isolated from autopsy tissues, with the exception of brain. Viral antigen in blood and tissues from patients with JUNV, MACV, SBAV, or GTOV infection can be usually detected by antigen-capture ELISA. Serologic diagnosis of AHF and BHF is usually made by IF and CF, but the limited specificity and sensitivity of these tests pose problems. The ELISA test is the most useful and practical for rapid detection of IgM and IgG antibodies in a clinical setting and sero-epidemiologic surveys. The plaque neutralization test can be valuable for evaluation of convalescent plasma units intended for therapeutic use in AHF.

Prevention and Control

Medical Management

Supportive therapy including hydration and pain relief management represent standard of care in suspected cases of mammarenaviral HFs. Platelet transfusions and factor replacement could help to control bleeding. Infusion of crystalloids to counteract the commonly observed moderate degree of vascular permeability should be carefully considered due to a high risk of pulmonary edema. The low cardiac output observed in human cases of AHF support the use of Swan-Ganz catheterization. LCMV ependymal infection and inflammation may cause acute hydrocephalus and a need for surgical shunting. Management of patients that undergo shock is difficult.

Patients with mammarenaviral HF pose in general a low risk of contagion. However, nosocomial outbreaks and infection of multiple contacts have occurred when the index case was severely ill with high viremia and viral load in tissues. Patients may excrete virus in urine or semen for weeks after recovery from disease. Therefore, body fluids should be monitored for infectivity before the patient is released, and counseling should be provided emphasizing protection of sexual partners and the use of disinfectant prior to use of toilets. The highest exposure risk is parenteral and can be minimized through staff training. Respiratory protection against small-particle aerosols should be implemented with caregivers and people in close proximity to patients. Special precautions are indicated when blood and other body fluids are handled in the clinical laboratory.

Antiviral Drugs

The prophylactic and therapeutic value of the nucleoside analog ribavirin (Rib) against human pathogenic mammarenaviruses is well supported by results from both cell culture and animal models of infection. Importantly, Rib reduced both morbidity and mortality in human cases of LF, and evidence suggests that Rib treatment can have also clinical benefits in cases of JUNV, MACV and SABV infections. However, the need of intravenous administration for optimal efficacy, poor penetration into the CSF, and side effects, including anemia and congenital disorders, pose some limitations to the use of Rib. In addition to Rib, several compounds have been reported to have anti-mammarenaviral activity in cultured cells, but their safety and efficacy in vivo remain to be determined. The broad-spectrum antiviral favipiravir and the mammarenaviral GP-mediated fusion inhibitor ST-193 have yielded very promising results in different animal models of mammarenaviral disease.

Progress in mammarenavirus molecular genetics has facilitated the development of screens to identify drugs targeting specific steps of the arenavirus lifecycle. These drugs include direct-acting antivirals (DAAs) that target specific viral gene products and functions, and drugs that target host-cell factors (HTAs) required for the completion of virus replication cycle. Combination therapy of DAAs and HTAs should counteract the emergence of drug resistant variants often observed with monotherapy strategies. Moreover, related viruses are likely to rely on the same host machinery, thus providing an opportunity for the development of broad-spectrum antiviral therapeutics.

Limited market opportunities are an obstacle for the development and licensing of new antiviral drugs against human pathogenic mammarenaviruses. It would be therefore beneficial to use the newly developed screening platforms to implement drug repurposing strategies that can significantly reduce the time and resources required to advance a candidate antiviral drug into the clinic. Moreover, drug repurposing can generate new knowledge on virus biology by uncovering previously unexplored pathways and specific host cell factors contributing to virus multiplication, knowledge that can be harnessed to identify new targets and therapeutics.

Antibody Therapy

Treatment of AHF cases with convalescent plasma from survivors of AHF has been shown to be quite effective in reducing AHF-associated mortality. About 10% of patients treated with convalescent plasma may exhibit usually self-limited neurologic signs including fever,

headache, cerebellar tremor, and cranial nerve palsies, which may reflect the prior invasion of the CNS by the virus. Studies in animal models of infection suggest that passive antibody therapy could be also helpful to treat BHF, VeHF and LF. Experimental studies of passive protection by monoclonal antibodies have been successful in LCMV. Notably, LASV GPC-specific recombinant human monoclonal antibodies derived from survivors of LF with strong neutralization profiles in cell-based assays have shown robust therapeutic efficacy in guinea pig and non-human primate (NHP) models of LF, supporting the feasibility of antibody-based therapy against LF.

Vaccines

The safety, immunogenicity, and protective efficacy of the JUNV live-attenuated Candid #1 strain was demonstrated in preclinical studies in both guinea pig and NHP models of AHF. Vaccination campaigns focused on agricultural workers in the JUNV-endemic area showed Candid #1 to be an effective and safe vaccine in humans, and it was licensed in 2006 for use exclusively in Argentina. However, comparison of Candid#1 clonal isolates from blood of vaccinated NHPs revealed a 1000-fold range of virulence among them, raising some concerns about the stability of the Candid #1 vaccine.

LASV is the mammarenavirus that poses the highest concern to human health due to morbidity and lethality associated with LF together with the vast LASV-endemic regions and size of the population at risk in Western Africa. Accordingly, LF has been included on the revised list of priority diseases of the WHO R&D Blueprint for which the development of a vaccine is encouraged. Studies involving LF survivors and animal models of LASV infection indicate that early and robust virus-specific $CD4^+$ and $CD8^+$ T cell responses, rather than the presence of neutralizing antibodies, which appear late after infection and at low titers, are the best correlates of recovery and protection. Although antibodies do not appear to contribute to viral control and recovery during acute LASV infection, genetically engineered neutralizing monoclonal antibodies can be successfully used for immunotherapy. These data suggest that an ideal LASV vaccine should trigger both long-term robust cell-mediated immunity (CMI) and humoral responses following a single immunization, features frequently displayed by live-attenuated vaccines (LAV). A number of LASV vaccine platforms have shown promising result in animal models of LASV, including LAV candidates based on vaccinia virus, vesicular stomatitis Indiana virus (VSIV), Mopeia virus (MOPV) an OW arenavirus closely related to LASV, yellow fever virus 17D vaccine strain, measles virus (MV)-based vector, alphavirus replicons and the ML29 reassortant carrying the L segment of the non-pathogenic MOPV and the S segment of LASV.

The Coalition for Epidemic Preparedness Innovations (CEPI) was created as non-profit organization to accelerate vaccine development against emerging epidemic infections for which the commercial market is insufficient to justify private investment. LASV vaccine candidates supported by CEPI include recombinant VSV and MV expressing LASV GPC and DNA-based vaccines. A single injection of rVSV/LASV-GPC experimental vaccine, in which VSIV G was replaced with LASV GPC, fully protected guinea pigs and NHPs against LASV strains from the same clade. Despite these promising results, there are still some safety concerns because of reported side effects in individuals vaccinated with an Ebola virus vaccine based on the same platform (rVSVΔG/EBOV-GP), which included post-vaccination arthritis, vector RNAemia, and detection of infectious vaccine virus in the skin of vaccinated individuals. The MV-based vector vaccine platform has shown promising results in human clinical trials providing antibody-based protection. Whether this platform would be effective for development of a vaccine against LASV with predominant T cell-mediated mechanism of protection remains to be determined. Currently there is no human licensed preventive DNA vaccine for any infectious disease, which poses great technical obstacles for developing an DNA-based vaccine against LASV.

The LASV candidate vaccine reassortant ML29 induced a robust cross-reacting and protective, sterilizing cell-mediated immune response against strains from distantly-related LASV lineages in a guinea pig model of LF. In addition, ML29 was shown to be stable and safe in animal models of LF, including immunocompromised NHPs. This finding is relevant because the LASV vaccine target population will likely include individuals with some degree of undiagnosed immune suppression due to the high prevalence of malaria and HIV-1 infections in Western Africa. Progress in arenavirus molecular genetics can also facilitate the implementation of novel strategies for the development of safe and effective LF vaccines based on the use of codon deoptimization and reorganization of the coding and non-coding intergenic regions.

Perspective

Since the discovery of LCMV more than 70 years ago, mammarenaviruses have served as important model systems for the study of host–virus interactions. Specifically, studies with LCMV in mice, the virus's natural reservoir, have uncovered a wide range of principles in the fields of virology and immunology that apply universally to other viral infections, including virus-induced immunopathology, MHC restriction, the contribution of negative immune regulators to viral persistence, and the concept of T cell exhaustion and demonstration that PD-1/PD-L1 blockade can rescue T cell function. In addition to their impact on basic science, mammarenaviruses include several human pathogens that remain a significant public health risk in much of the world. Control of human pathogenic mammarenaviruses faces significant obstacles including current limited diagnostic protocols and sparse resource allocation for vaccine development and other medical countermeasures. However, progress in molecular genetics has facilitated novel approaches for the investigation of mammarenavirus molecular and cell biology, whereas the application of next generation sequencing technologies has brought a major breakthrough to the investigation of mammarenavirus epidemiology including their prevalence and distribution, as well as genetic diversity. This new knowledge has had also great implications for the development of new approaches to antivirals and vaccines to combat human pathogenic mammarenaviruses.

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